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Introduction

Key to the kit is our proprietary DNA binding systems that allow the high efficient binding of DNA to our ezBind™ matrix while proteins and other contaminates are removed under certain optimal conditions. Nucleic acids are easily eluted with sterile water or elution buffer.

Unlike other procedures, our patented plasmid purification kit has no guanidine salt in the buffer, the purified DNA is guanidine/ion exchange resin residues free which enable the high performance of downstream applications such as transfection, restriction mapping, library screening, sequencing, as well as gene therapy and genetic vaccinations.

Important Notes

Plasmid Copy Numbers: The yield of plasmid DNA depends on the origin of the replication and the size of the plasmid. The protocols are optimized for high copy number plasmid purification. For low copy number plasmids, both the culture volume and the buffer volume need to be scaled up 2 times. Reference Table 1 for the commonly used plasmids,

Table 1 Commonly used plasmids.

Plasmid	Origin	Copy Numbers	Expected Yield (μg per 500 mL)
pSC101	pSC101	5	50-60
pACYC	P15A	10-12	80-100
pSuperCos	pMB1	10-20	80-150
pBR322	pMB1	15-20	100-150
pGEM®	Muted pMB1	300-400	2000-2500
pBluescript®	ColE1	300-500	2000-3000
pUC	Muted pMB1	500-700	3000-4000

Host Strains: The strains used for propagating plasmid have significant influence on yield. Host strains such as Top 10 and DH5a yield high-quality plasmid DNA. *endA+* strains such as JM101, JM110, HB101, TG1 and their derivatives, normally have low plasmid yield due to either endogenous endonucleases or high carbohydrates released during lysis. We recommend transform plasmid to an *endA-* strain if the yield is not satisfactory. For purifying plasmid DNA from *endA+* strains (Table 2), we recommend use product PD1714.

Table2 *endA* strains of *E. Coli*.

EndA*- Strains of *E. Coli							
DH5α	DH1	DH21	JM106	JM109	SK2267	SRB	XLO
TOP10	DH10B	JM103	JM107	SK1590	MM294	Stbl2™	XL1-Blue
BJ5182	DH20	JM105	JM108	SK1592	Select96™	Stbl4™	XL10-Gold
EndA*+ Strains of *E. Coli							
C600	JM110	RR1	ABLE® C	CJ236	KW251	P2392	BL21(DE3)
HB101	TG1	TB1	ABLE® K	DH12S™	LE392	PR700	BL21(DE3) pLysS
JM101	JM83	TKB1	HMS174	ES1301	M1061	Q358	BMH 71-18
All NM strains				All Y strains			

Optimal Cell Mass (OD_{600} x mL of Culture): This procedure is designed for isolating plasmid grown in standard LB medium (Luria Bertani) for 12 -16 hours to a density of OD_{600} 2.0 to 3.0. If rich medium such as TB or 2xYT are used, make sure the cell density doesn't exceed 3.0 (OD_{600}). A high ratio of biomass over lysis buffers result in low DNA yield and purity.

Culture Volume: Use a flask or tube with a volume at 4 times the culture medium to secure optimal condition for bacteria growth. Don't exceed the maximum culture volume suggested in the protocol. Incomplete lysis due to over amount of bacterial culture results in lower yield and less purity.

Table 3 The optimal cell mass, culture Volume and Binding Capacity for the mega DNA units,

DNA Units	Mega 3	Mega 6	Mega 10
Optimal Cell Mass	1200	2500	4500
Culture Volume	500 mL	1000 mL	1500 mL
Binding Capacity	3-4 mg	6-7 mg	10-12 mg

Storage and Stability

Buffer A1 should be stored at 4℃ once RNase A is added. All other materials can be stored at room temperature (22-25°C). The Guaranteed shelf life is 12 months from the date of purchase.

Before Starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps.

Important:

- ☑ **RNase A**: It is stable for more than half a year when stored at room temperature. Spin down RNase A vial briefly. Add the RNase A solution to Buffer A1 and mix well before use.
- ☑ Buffer ER should be stored at 4℃.
- ☑ Buffer B1 precipitates below room temperature. It is critical to warm up the buffer at 50℃ to dissolve the precipitates before use.
- ☑ Buffer N3 may form precipitates below 10℃, warm up at 37℃ to dissolve the precipitates before use.
- ☑ Keep the cap tightly closed for Buffer B1 after use.
- ☑ Make sure the availability of centrifuge and vacuum manifold, especially, after mixing the lysate with ethanol, the sample needs to be processed immediately by vacuum.

Materials supplied by users:

- ☑ 70% ethanol and 100% ethanol.
- ☑ Pump-driven vacuum system, 1,000 mL bottle (Corning# 430518 or 430282) or equivalent pyrex glass bottles.
- ☑ 50 mL conical tubes.
- ☑ High speed centrifuge tube for endotoxin removal if desired.

Kit Contents

Catalog#	PD1621-00	PD1621-01	PD1621-02
Preps	1	2	10
DNA Unit	1	2	10
Filter Unit	1	2	10
Replacement Cup	1	2	10
Buffer A1	35 mL	70 mL	350 mL
Buffer ER	1.8 mL	3.5 mL	17.5 mL
Buffer B1	35 mL	70 mL	350 mL
Buffer D1	3.5 mL	7 mL	35 mL
Buffer N3	10 mL	20 mL	100 mL
Buffer RET	65 mL	130 mL	2×330 mL
RNase A(20 mg/mL)	3.5 mg (175 μL)	7.0 mg (350 μL)	35 mg (1.75 mL)
Endofree Elution Buffer	20 mL	40 mL	200 mL
User Manual	1	1	1

Safety Information

- Buffer N3 contains acetic acid, use gloves and protective eyewear when handling.
- Buffer N3, Buffer RET contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste.

EZgene™ Plasmid ezFilter Megaprep 3 Protocol

1. Inoculate **500 mL** LB containing appropriate antibiotic with 500 µL fresh starter culture. Grow at 37 ℃ for 14-16 h with vigorous shaking.

   Note: The best way to prepare a starter culture: Inoculate a single colony from a freshly grown selective plate into 1 mL LB medium containing the appropriate antibiotic and grow at 37 ℃ for 6-8 h with vigorous shaking (~250 rpm). The buffer volumes need to be scaled up if processing over 500 mL of culture.

2. Harvest **500 mL** overnight bacterial cells by centrifugation at 5,000 x g for 10 minutes at room temperature. Decant or aspirate medium and discard.

   Note: Remove the residual medium completely for optimal cell lysis and neutralization.

3. Resuspend the bacterial pellet in **30 mL Buffer A1** (Add **RNase A** to **Buffer A1** before use). Pipet or vortex till the bacterial pellet dispersed thoroughly (Complete resuspension is critical for optimal yields). Then add **1.5 mL Buffer ER** into the suspended bacterial culture. Mix well by inverting 5-10 times.

4. Add **27 mL Buffer B1.** Mix gently but thoroughly by inverting 10 times and incubate at room temperature for 5 minutes to obtain a cleared lysate. Do not incubate longer than 5 minutes. Over-incubating causes genomic DNA contamination and plasmid damage. Avoid vigorous mixing as this will shear the genomic DNA. Then add **3 mL Buffer D1**, mix gently and incubating for another 5 minutes

5. Add **8 mL Buffer N3** and mix immediately by inverting 5 times till a flocculent white precipitate forms. Vortex the lysate for 5 seconds.

   Note: It is critical to mix the lysate well, if the mixture still appears conglobated, brownish or viscous, more mix is required to completely neutralize the solution.

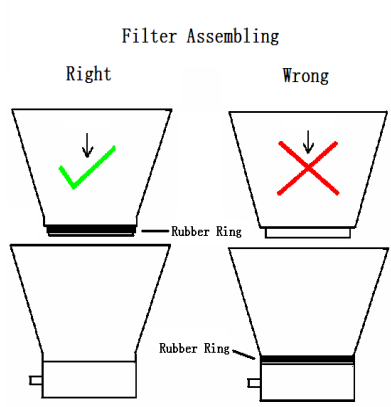
6. Attach the 2-layer filter unit to a sterile 500 mL or 1000 mL standard bottle (Corning# 430518 or 430282 or equivalent pyrex glass bottle) and screw tight. Connect the unit to a pump-driven vacuum system.

7. Transfer the clear lysate from the bottom of the mixture (use a 50 mL serological pipet) to the filter unit. Stand by for 5 minutes and turn on the vacuum with low vacuum force and increase to maximum vacuum force after 5 minutes.

   Note 1: Low vacuum force prevents clogging of the filter membranes.
   Note 2: Use a 50 mL serological pipet to transfer the relatively clear lysate from the

bottom of the lysate bottle to the filter unit. This will speed up the flow rate of the filter unit. Normally around 200 mL lysate can be filtered through the filter unit within 20-30 minutes. Pour the remaining white precipitates to the filter unit when most of the lysate has been filtered through.

Figure 1. Instruction of filter assembling.



Note 3: If the flow through gets too slow, turn off the vacuum and wait for 1 minute. Carefully detach the upper filter cup and replace it with the replacement cup. Assemble the unit as **Figure 1.** Pour the lysate from the original cup to the replacement cup. Turn on the vacuum and filter the rest of the lysate.

8. When most of the lysate has been filtered through the unit, turn off the vacuum, wait for 1 minute, detach the unit and discard the upper filter cup including the rubber rings.

Note: The DNA is in the collection bottle.

9. Connect the DNA unit to a 500 mL or 1,000 mL standard bottle and screw tight. Connect the DNA unit to the vacuum with the vacuum off. Add **1 volume** of **Buffer RET** (For example, **60 mL** of **Buffer RET** to **60 mL** of **clear lysate)**, and add **36 mL 100% ethanol** to the lysate bottle. Mix well by sharp hand shaking 3-5 times and immediately pour **half** of the **lysate/ethanol mixture** to the DNA unit and turn on the vacuum.

10. Pour the rest of the **lysate/ethanol mixture** into the DNA unit. When all the lysate pass through the DNA unit, vacuum for 1 minute.

11. Wash the DNA membrane with **50 mL 70% ethanol** and vacuum for 1 minute at maximum force. Wash the DNA membrane with another **50 mL 70% ethanol** and vacuum for 1 minute at maximum force.

12. Add **50 mL 100% ethanol** evenly to the DNA membrane and vacuum for 1 minute. Turn off the vacuum, wait for 1 minute, and discard the liquid waste in the bottle. Reconnect the bottle to the DNA binding unit. Turn on the vacuum

for 20 minutes at maximum force ***(It is critical to dry the residual ethanol for optimal yield).***

13. Turn off the vacuum, wait for 1 minute, and replace the 500 mL or 1,000 mL standard bottle with a sterile 50 mL conical tube, screw tight.

14. Add **10 mL Endofree Elution Buffer** evenly to the membrane and incubate for 2 minutes. Turn on vacuum to elute DNA. Typically **3~5 mL** of solution can be collected. This is the 1st elution.

15. Turn off the vacuum and replace the 50 mL conical tube with another sterile 50 mL conical tube, screw tight. Add **5 mL Endofree Elution Buffer** and incubate for 1 minute. Turn on the vacuum and collect the 2nd elution, typically **2 ~4 mL** of solution can be collected.

Note: The DNA is ready for downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.
Note: Two elutions give rise to maximum DNA yield. For maximum yield and higher concentration, pool the elutions together, add 0.1 volume 3M Potassium Acetate or Sodium acetate (pH 5.2) and 0.7 volume isopropanol. Centrifuge at top speed for 10 min. Discard supernatant. Wash the DNA with 1000 µL 70% ethanol, centrifuge for 5 min, carefully decant. Air-dry the pellet for 10-20 minutes in a tissue culture hood. Resuspend the DNA in Endofree Elution Buffer.
Note: Use less Endofree Elution Buffer if high concentration is desired.

DNA concentration (µg/mL) = OD_{260} nm x 50 x dilution factor.

Purification of Low-Copy-Number Plasmid and Cosmid

The yield of low copy number plasmid is normally around 0.1 – 1 μg /mL of overnight culture. For isolating low copy number or medium copy number plasmid DNA, use the following guideline:

1. Culture volume: Use **2 x volume** of the high copy number culture

2. Use **2 x volume** of the **Buffer A1, Buffer B1 and Buffer N3 and 100% ethanol**. Additional buffers can be purchased from Biomiga.

3. Use **same volume** of **wash buffer (70% ethanol and 100% ethanol)** and **Endofree Elution Buffer**.

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Low Yield	Poor Cell lysis.	• Resuspend pellet thoroughly by votexing and pipeting prior adding Buffer B1. • Make fresh buffer B1 if the cap had not been closed tightly. (Buffer B1: 0.2N NaOH and 1%SDS).
Low Yield	Bacterial culture overgrown or not fresh.	Grow bacterial 12-16 hours. Spin down cultures and store the pellet at -20℃ if the culture is not purified the same day. Do not store culture at 4℃ over night.
Low Yield	Low copy-number plasmid.	Increase culture volume to 2 x of original volume. Increase the volume of buffer A1, B1, N3 according to instruction on page 8.
No DNA	Plasmid lost in Host E. coli.	Prepare fresh culture.
Genomic DNA contamination	Over-time incubation after adding buffer B1.	Do not vortex or mix aggressively after adding buffer B1. Do not incubate more than 5 minutes after adding solution B1.
RNA contamination	RNase A not added to Buffer A1.	Add RNase A to Buffer A1.
Plasmid DNA floats out of wells while running in agarose gel, DNA doesn't freeze or smell of ethanol	Ethanol traces not completely removed from column.	Make sure that no ethanol residual remaining in the silicon membrane before eluting the plasmid DNA. Re-vacuum again if necessary.